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(54) Method of producing L-glutamic acid by fermentation.

(57) The present invention provides a low-cost and efficient production method of L-glutamic acid, by improving the productivity of L-glutamic acid in a microorganism of the genus Escherichia.
[Constitution]

A method of producing L-glutamic acid by fermentation comprising culturing in a liquid culture medium a mutant of the genus Escherichia having L-glutamic acid-producing ability whose α -ketoglutarate dehydrogenase activity is deficient or reduced and phosphoenolpyruvate carboxylase and glutamate dehydrogenase activities are amplified, accumulating L-glutamic acid in the culture, and recovering L-glutamic acid therefrom.

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Field of the Invention

The present invention relates to a mutant useful for producing L-glutamic acid by fermentation as well as a method of producing L-glutamic acid by fermentation using such a mutant. L-glutamic acid is an amino acid widely used as an additive for foods and in medicaments.

Prior Art

L-glutamic acid has conventionally been produced by fermentation using glutamic acid-producing bacteria and mutants thereof such as those of the genus Brevibacterium, Corynebacterium or Microbacterium (Amino acid fermentation, Gakkai Shuppan Center, pp.195 to 215 (1986)). Other known methods of producing L-glutamic acid by fermentation include a method employing microorganisms of the genus Bacillus, Streptomyces or Penicillium (US Patent No. 3,220,929) and a method employing microorganisms of the genus Pseudomonas, Arthrobacter, Serratia or Candida (US Patent No. 3,563,857). Even though such conventional methods produce significantly large amounts of L-glutamic acid, an even more efficient and less expensive method of producing L-glutamic acid is desired in order to meet the ever-increasing demand.

Escherichia coli is a potentially excellent L-glutamic acid-producing bacterium in view of its high growth rate and the availability of sufficient gene information, while the reported amount of L-glutamic acid production by Escherichia coli is as low as 2.3 g/l (J. Biochem., Vol. 50, pp.164 to 165 (1961)). Recently, a mutant exhibiting a deficient or reduced α -ketoglutarate dehydrogenase (hereinafter referred to as α -KGDH) was reported to have the ability to produce large amounts of L-glutamic acid (French Patent Application Laid-Open No. 2680178).

Problems to be Solved by the Invention

An objective of the present invention is to enhance the L-glutamic acid-producing ability of strains belonging to the genus Escherichia and to provide a method of producing L-glutamic acid more efficiently and at a lower cost.

Means to Solve the Problems

Now it has been found surprisingly in our study on the production of L-glutamic acid by mutants of Escherichia coli that a mutant whose α -KGDH activity is deficient or reduced, and whose phosphoenolpyruvate carboxylase (hereinafter referred to as PPC) and glutamate dehydrogenase (hereinafter referred to as GDH) activities are enhanced, has a high L-glutamic acid-producing ability, and thus the present invention has been accomplished.

Accordingly, the present invention relates to :

A mutant of the genus Escherichia having L-glutamic acid-producing ability whose α -KGDH activity is deficient or reduced, and PPC and GDH activities are enhanced; and,

A method of producing L-glutamic acid by fermentation comprising culturing in a liquid culture medium a mutant of the genus Escherichia having L-glutamic acid-producing ability whose α -KGDH activity is deficient or reduced and PPC and GDH activities are enhanced, accumulating L-glutamic acid in the culture, and recovering L-glutamic acid therefrom.

The present invention is described in more detail below.

(1) Derivation of a mutant of the genus Escherichia exhibiting deficient or reduced α -KGDH activity

As a starting parent strain to be used for preparing the present mutant, any non-pathogenic strain of the genus Escherichia may be employed. Examples of such strains are listed below.

Escherichia coli K-12 (ATCC 10798)
Escherichia coli W3110 (ATCC 27325)
Escherichia coli B (ATCC 11303)
Escherichia coli W (ATCC 9637)

A mutant of the genus Escherichia which has L-glutamic acid-producing ability and having deficient or reduced α -KGDH activity may be prepared as follows.

The starting parent strain mentioned above is first exposed to X-radiation or ultraviolet light or mutagenic agents such as N-methyl-N'-nitro-N-nitrosoguanidine (hereinafter referred to as NG) to introduce the mutation.

Alternatively, gene engineering technology, for example, gene recombination, gene transformation or cell

fusion, may be used to efficiently introduce the intended mutation.

A method of obtaining an α -KGDH-deficient mutant by means of gene recombination is conducted as follows. Based on the known nucleotide sequence (Euro. J. Biochem. Vol. 141, pp. 351 to 359 (1984)) of α -ketoglutarate dehydrogenase gene (hereinafter referred to as sucA gene), primers are synthesized and then the sucA gene is amplified by the PCR method using the chromosomal DNA as a template. Into the amplified the sucA gene, a drug-resistant gene is inserted to obtain a sucA gene whose function is lost. Subsequently, using homologous recombination, the sucA gene on the chromosome is replaced by a sucA gene whose function is lost by means of the insertion of the drug-resistant gene.

After subjecting the parent strain to mutagenic treatment, the intended mutants may be screened by procedures as illustrated below.

A mutant exhibiting a deficient or reduced α -KGDH activity is either not able to grow or is able to grow only at a significantly reduced growth rate in a minimum culture medium containing glucose as the carbon source under aerobic condition. However, even under such condition, normal growth is possible by adding succinic acid or lysine plus methionine to the minimum culture medium containing glucose. On the other hand, anaerobic condition allows the mutant to grow even in the minimum culture medium containing glucose (Molec. Gen. Genetics, Vol. 105, pp. 182 to 190 (1969)). Based on these findings, the desired mutants can be screened.

The following strain is an example of the mutants thus obtained whose α -KGDH activity is deficient or reduced and which are listed below.

Escherichia coli W3110 sucA::Kmr

A mutant whose α -KGDH activity is deficient or reduced is more useful in view of its enhanced ability to produce L-glutamic acid when it further has the properties that L-glutamic acid-degrading activity is reduced or the expression of ace operon, that is, malate synthase (aceB) - isocitrate lyase (aceA) - isocitrate dehydrogenase kinase/phosphatase (aceK) operon becomes constitutive. These properties are discussed in French Patent Application Laid-open No. 2680178. As a matter of course, properties already known to be effective for improving L-glutamic acid-productivity, such as various types of auxotrophy, antimetabolite resistance and antimetabolite sensitivity, are also desirable for enhancing L-glutamic acid production ability.

A mutant having reduced ability to degrade L-glutamic acid may be isolated as a mutant which either cannot grow or can grow only slightly in a minimum culture medium containing L-glutamic acid as the sole carbon source instead of glucose or containing L-glutamic acid as a sole nitrogen source instead of ammonium sulfate. However, as a matter of course, when an auxotroph is employed for the derivation, the minimum essential amount of the nutrient required for the growth may be added to the culture medium.

A mutant in which the expression of the ace operon is constitutive may be obtained as a strain whose parent strain is a phosphoenolpyruvate synthase-deficient strain and which can grow in a minimum culture medium containing lactic acid as the carbon source but cannot grow in a minimum culture medium containing pyruvic acid or acetic/pyruvic acid as the carbon source, or as a strain which shows a higher growth rate than that of its parent strain whose α -KGDH is deficient or reduced under aerobic condition (J. Bacteriol., Vol. 96, pp. 2185 to 2186 (1968)).

Examples of the mutants described above are as follows.

Escherichia coli AJ 12628 (FERM BP-3854)

Escherichia coli AJ 12624 (FERM BP-3853)

Escherichia coli AJ 12628 is a mutant having a reduced α -KGDH activity and a reduced ability to degrade L-glutamic acid in combination with constitutive expression of ace operon. Escherichia coli AJ 12624 is a mutant having reduced α -KGDH activity and a reduced ability to degrade L-glutamic acid (French Patent Application Laid-open No. 2680178).

In the mutant thus obtained which exhibits deficient or reduced α -KGDH activity, the flow of biosynthesis of L-glutamic acid via α -ketoglutaric acid in the TCA cycle is improved, resulting in an enhanced ability of producing L-glutamic acid. Also the productivity of L-glutamic acid is increased in the mutant exhibiting deficient or reduced α -KGDH activity and significantly low ability to degrade the produced L-glutamic acid or in the mutant further having a constitutive expression of the ace operon whereby the growth is improved.

(2) Derivation of a mutant of the genus Escherichia having amplified PPC activity and GDH activity

In the examples described below, a mutant of the genus Escherichia having amplified PPC and GDH activities was obtained from a starting parent strain exhibiting deficient or reduced α -KGDH activity and having the ability to produce L-glutamic acid. It is also possible to use a wild strain of the genus Escherichia as the parent strain to obtain a mutant having amplified PPC and GDH activities whereafter a mutant is bred which exhibits deficient or reduced α -KGDH activity.

Accordingly, the starting parent strain used to prepare a mutant having amplified PPC and GDH activities

is preferably a mutant of the genus Escherichia whose α -KGDH activity is deficient or reduced and which has the ability to produce L-glutamic acid or a non-pathogenic wild type strain of the genus Escherichia. Examples of such strains are listed below.

- 5 Escherichia coli W3100 sucA::Kmr
- Escherichia coli AJ 12628 (FERM BP-3854)
- Escherichia coli AJ 12624 (FERM BP-3853)

(Those listed above are the mutants of the genus Escherichia whose α -KGDH activity is deficient or reduced and which have the ability to produce L-glutamic acid.)

- 10 Escherichia coli K-12 (ATCC 10798)
- Escherichia coli W3110 (ATCC 27325)
- Escherichia coli B (ATCC 11303)
- Escherichia coli W (ATCC 9637)

(Those listed above are the non-pathogenic wild strains of the genus Escherichia.)

In order to amplify PPC and GDH activities, the genes coding for PPC and GDH are cloned in an appropriated plasmid, which is then used to transform the starting parent strain employed as a host. The copies of the genes coding for PPC and GDH (hereinafter referred to as ppc gene and gdhA gene, respectively) in the transformed cells are increased, resulting in amplified PPC and GDH activities.

The ppc gene and gdhA gene to be cloned may be cloned into a single plasmid to be introduced into the starting parent strain employed as the host, or may be cloned separately into two types of plasmid which are compatible in the starting parent strain.

Alternatively, the amplification of PPC and GDH activities may be conducted by allowing the ppc and gdhA genes to be present as multicopies on the chromosomal DNA of the starting parent strain employed as the host. In order to introduce the ppc and gdhA genes as multicopies into the chromosomal DNA of the genus Escherichia, homologous recombination is applied utilizing a target sequence present as a multicopy on the chromosomal DNA. The sequence present as the multicopy may be a repetitive DNA and an inverted repeat present at the terminal of insertion sequence. Alternatively, as described in Japanese Patent Application Laid-open No. 2-109985, the ppc and gdhA genes are cloned on a transposon, which is then transposed, thereby introducing the multicopy into the chromosomal DNA. The copies of the ppc and gdhA genes in the transformed cells are increased, resulting in the amplification of PPC and GDH activities.

In addition to the gene amplification described above, the amplification of PPC and GDH activities may also be conducted by replacing the promoters of the ppc and gdhA genes with those having higher potencies. For example, lac promoter, trp promoter, trc promoter, tac promoter, P_R promoter and P_L promoter of a lambda phage are known to be strong promoters. By enhancing the expression of the ppc gene and of the gdhA gene, the PPC and GDH activities are amplified.

The ppc and gdhA genes can be obtained by isolating the genes which are complementary with regard to auxotrophy of the mutants which are either PPC or GDH deficient. Alternatively, since the nucleotide sequences of these genes of Escherichia coli are known (J. Biochem., Vol. 95, pp. 909 to 916 (1984); Gene, Vol. 27, pp. 193 to 199 (1984)), the primers are synthesized based on the nucleotide sequences and then the genes are obtained by the PCR method using the chromosomal DNA as the template.

(3) Production of L-glutamic acid by fermentation using a mutant of the genus Escherichia capable of producing L-glutamic acid which exhibits deficient or reduced α -KGDH activity and has amplified PPC and GDH activities

For the purpose of producing L-glutamic acid by fermentation using a mutant of the genus Escherichia capable of producing L-glutamic acid which exhibits deficient or reduced α -KGDH activity and has amplified PPC and GDH activities, a standard culture medium containing carbon sources, nitrogen sources, inorganic salts and, if necessary, organic trace nutrients such as amino acids and vitamins and a standard culture method may be employed. The carbon sources and the nitrogen sources employed in the culture medium may be any of those catabolized by the mutant employed.

The carbon sources may be saccharides such as glucose, glycerol, fructose, sucrose, maltose, mannose, galactose, starch hydrolysate and molasses, and organic acids such as acetic acid and citric acid may also be employed independently or in combination with other carbon sources.

The nitrogen sources may be ammonia and ammonium salts such as ammonium sulfate, ammonium carbonat , ammonium chloride, ammonium phosphate, ammonium acetate as well as nitrates.

The organic trace nutrients may be amino acids, vitamins, fatty acids and nucleic acids as they are or as contained in peptone, casamino acid, yeast extract, soy protein hydrolysate and the like. In cases of using an auxotroph the nutrient required for its growth should be supplemented.

The inorganic salts may be phosphate, magnesium salts, calcium salts, iron salts, manganese salts and the like.

Cultivation is conducted at a fermentation temperature from 20 to 45°C at a pH controlled to be in a range of from 5 to 9 with aeration. When the pH is controlled during the cultivation, calcium carbonate or alkali such as ammonia gas may be added for neutralization. After culturing for from 10 hours to 4 days, a significant amount of L-glutamic acid is accumulated in the culture medium.

L-glutamic acid in the culture medium after cultivation may be recovered by any of the known methods. For example, the cells are removed from the culture medium, which is then concentrated and precipitated or subjected to ion exchange chromatography to obtain L-glutamic acid.

10 Brief Description of the Drawings

- Fig. 1 shows the construction procedure of pBR-sucAB,
- Fig. 2 shows a procedure for disrupting the sucA gene on the chromosomal DNA of Escherichia coli W3110,
15 and
- Fig. 3 shows the construction procedure of pGK.

Examples

20 The present invention is further described by the following examples.

Example 1

(1) Cloning of sucA gene and dihydrolipoamide succinyl transferase gene of Escherichia coli

25 The nucleotide sequences of sucA gene and dihydrolipoamide succinyl transferase gene (hereinafter referred to as sucB gene) of Escherichia coli K12 are known. The known nucleotide sequences of sucA gene and sucB gene are disclosed in Euro. J. Biochem., Vol. 141, pp. 351 to 374 (1984), and also shown here as Sequ ID No. 7 in the sequence listing. The nucleotide sequence from the 327th through the 3128th base residues corresponds to ORF (open reading frame) of the sucA gene, while that from the 3143rd through the 4357th base residues corresponds to ORF of the sucB gene. According to the nucleotide sequences reported, primers shown in Sequ ID No. 1 to 4 were synthesized and sucA and sucB genes were amplified by PCR method employing the chromosomal DNA of Escherichia coli W3110 as a template.

30 The synthetic primers used to amplify the sucA gene had the nucleotide sequences shown in Sequ ID No. 1 and 2, and Sequ ID No. 1 corresponds to the sequence consisting of the 45th through the 65th base residues in the nucleotide sequence figure of the sucA gene described in Euro. J. Biochem., Vol. 141, p. 354 (1984). It also corresponds to the sequence consisting of the 45th through the 65th base residues of the nucleotide sequence shown as Sequ ID No. 7.

35 Sequ ID No. 2 corresponds to the sequence consisting of the 3173rd through the 3193rd base residues in the nucleotide sequence figure of the sucB gene shown in Euro. J. Biochem., Vol. 141, p. 364 (1984). It also corresponds to the sequence consisting of the 3173rd through the 3193rd base residues of the nucleotide sequence shown as Sequ ID No. 7.

40 The synthetic primers used to amplify the sucB gene had the nucleotide sequences shown in Sequ ID No. 3 and 4, and Sequ ID No. 3 corresponds to the sequence consisting of the 2179th through 2198th base residues in the nucleotide sequence figure of the sucA gene shown in Euro. J. Biochem., Vol. 141, p. 354 (1984). It also corresponds to the sequence consisting of the 2179th through the 2198th base residues of the nucleotide sequence shown as Sequ No. 7.

45 Sequ ID No. 4 corresponds to the sequence consisting of the 4566th through the 4591st base residues in the nucleotide sequence figure of the sucB gene shown in Euro. J. Biochem., Vol. 141, p. 364 (1984). It also corresponds to the sequence consisting of the 4566th through the 4591st base residues of the nucleotide sequence shown as Sequ ID No. 7. The sucA gene and the sucB gene form an operon.

50 The chromosomal DNA of Escherichia coli W3110 was recovered by a standard method (Seibutsukogaku Jikkensho, Ed. by Nippon Seibutsu Kogaku Kai, pp. 97 to 98, Baifukan (1992)).

55 The PCR reaction was carried out under the standard conditions described on page 8 of PCR Technology (Ed. by Henry Erlich, Stockton Press (1989)).

Both ends of PCR products thus produced were converted into blunt ends using T4 DNA polymerase and cloned into a vector pBR322 at the EcoRV site. The plasmid obtained by cloning the sucA gene into pBR322 was designated as pBR-sucA, and that constructed with sucB was designated as pBR-sucB. The plasmids

thus obtained were introduced into Escherichia coli JM109 and the plasmids were prepared. Then the restriction maps were constructed and compared with the restriction maps of the sucA and sucB genes reported, thereby confirming that the genes which had been cloned were the sucA and sucB genes.

As shown in Fig. 1, pBR-sucB was digested with KpnI and EcoRI to prepare a DNA fragment containing the sucB gene. pBR-sucA was digested with KpnI and EcoRI to prepare a large fragment. Both fragments were ligated using T4 DNA ligase to produce pBR-sucAB.

(2) Disruption of the sucA gene on chromosomal DNA of Escherichia coli W3110

Fig. 2 outlines the disruption of the sucA gene on the chromosomal DNA of Escherichia coli W3110. pBR-sucAB was digested with KpnI and T4 DNA polymerase was used to obtain blunt ends. On the other hand, pUC4K (purchased from Pharmacia) was digested with PstI to prepare a DNA fragment containing a kanamycin-resistance gene, which was converted to have blunt ends using T4 DNA polymerase. Both fragments were ligated using T4 DNA ligase to obtain pBR-sucA::Kmr'. From this plasmid, a HindIII-EcoRI fragment containing the kanamycin-resistance gene was cut out as a linear DNA, which was used to transform Escherichia coli JC7623 (thr-1, ara-14, leuB6, Δ(gpt-proA)62, lacY1, tsx-23, supE44, galK2, λ-, rac-, sbcB15, hisG4, rbfD1, recB21, recC22, rpsL31, kdgK51, xyl-5, mtl-1, argE3, thi-1) obtained from the Escherichia coli Genetic Stock Center (at Yale University, USA), and strains in which the sucA gene on the chromosomal DNA was replaced with the sucA gene into which the kanamycin-resistance gene had been inserted (sucA::Kmr') were screened on L medium (bactotrypton 10 g/l, yeast extract 5 g/l, NaCl 5 g/l, agar 15 g/l, pH 7.2) supplemented with 25 µg/ml of kanamycin. Since Escherichia coli JC7623 possessed recB-, recC- and sbcB-, recombination can be achieved at a high frequency even if the transformation is conducted using a linear DNA.

From each of twelve (12) kanamycin-resistant strains thus obtained, the chromosomal DNA was prepared and digested with KpnI. By southern hybridization using a DNA fragment containing the sucA gene as a probe, all of 12 strains were confirmed to be strains in which the sucA gene on the chromosomal DNA was replaced with the sucA gene into which kanamycin-resistance gene had been inserted. While a wild strain exhibits two bands at 5.2 Kb and 6.2 Kb due to the presence of KpnI site in the DNA fragment containing the sucA gene when a 2.6 Kb EcoRI-HindIII fragment containing the sucA gene of pBR-sucA was used as the probe in the southern hybridization, strains having the replacement with sucA gene into which kanamycin-resistance gene has been inserted exhibits only one band at 11.4 Kb due to the disruption of the KpnI site upon introduction of the kanamycin-resistance gene. The kanamycin-resistance Escherichia coli JC7623 (sucA::Kmr') thus obtained was then infected with P1 phage and the phage lysate was prepared. Then Escherichia coli W3100 strain was transduced with the sucA::Kmr'. Transduction with P1 phage was conducted by a standard method (Seibutsu-kogaku Jikkensho, Ed. by Nippon Seibutsu Kogaku Kai, pp. 75 to 76, Baifukan (1992)). The representative of the kanamycin-resistance strains isolated was designated as W3110 sucA::Kmr'.

The α-KGDH activities of the strain W3110 sucA::Kmr' and Escherichia coli W3110 were determined according to the method by Reed et al (Methods in Enzymology, Vol. 13, pp. 55 (1969)). The results are shown in Table 1. α-KGDH activity of Escherichia coli W3110 sucA::Kmr' was not detected. Thus, Escherichia coli W3110 sucA::Kmr' is a strain deficient in α-KGDH activity.

Table 1

	W3110	W3110sucA::Kmr'
α-KGDH activity	3.70	Not detected
(Unit : micromoles/mg protein/min)		

(3) Cloning of gdhA gene of Escherichia coli W3110

Similarly as in the cloning of the sucA and sucB genes, the PCR method was used to clone the gdhA gene. According to the nucleotide sequence of gdhA gene reported by Fernando et al, primers for PCR were synthesized. The nucleotide sequence of the gdhA gene is disclosed in Gene, Vol. 27, pp.193 to 199 (1984), and is also shown here as Sequ ID No. 8 in the sequence listings. The nucleotide sequences of the primers are shown in Sequ ID Nos. 5 and 6.

Sequ ID No. 5 corresponds to the sequence from the 191st through the 171st base residues in the nucleotide sequence figure of gdhA gene shown in Gene, Vol. 27, p.195 (1984), and it also corresponds to the

sequence from the 3rd through the 23rd base residues in Sequ ID No. 8.

Sequ ID No. 6 corresponds to the sequence consisting of the 1687th through the 1707th base residues in the nucleotide sequence figure of the gdhA gene shown in Gene, Vol. 27, p.195, (1984), and it also corresponds to the sequence consisting of 1880th through the 1900th base residues in Sequ ID No. 8.

Using the synthetic primers the gdhA gene was amplified by the PCR method employing the chromosomal DNA of Escherichia coli W3110 as a template. PCR products thus obtained were purified and converted to have blunt ends using T4 DNA polymerase, and then ligated to pBR 322 digested with EcoRV to obtain a plasmid pBRGDH.

10 (4) Construction of a plasmid having the ppc and gdhA genes

Fig. 3 shows the procedure for the construction of a plasmid having the ppc and gdhA genes. The plasmid pS2 in which 4.4Kb Sall fragment containing the whole region of the ppc gene derived from Escherichia coli K-12 was inserted into the Sall site of pBR322 (J. Biochem, Vol. 9, pp.909 to 916 (1984)) was digested with HindIII and both ends were made blunt using T4 DNA polymerase. On the other hand, a DNA fragment containing the gdhA gene synthesized by the PCR method was converted to have blunt ends using T4 DNA polymerase. Subsequently, both fragments were ligated using T4 DNA ligase. The plasmid thus obtained was used to transform a GDH deficient strain, Escherichia coli PA 340 (thr-1, fhuA2, leuB6, lacY1, supE44, gal-6, λ -, gdh-1, hisG1, rfbD1, galP63, Δ (gltB-F), rpsL19, malt1(lambdaR), xy1-7, mtl-2, argH1, thi-1) obtained from the Escherichia coli Genetic Stock Center (at Yale University, USA) and an ampicillin-resistant strain which had lost its glutamic acid requirement for growth was isolated. From this strain, a plasmid was prepared and the restriction map was constructed, whereby it was confirmed that the ppc and gdhA genes were present on this plasmid. This plasmid was designated as pGK.

25 (5) Introduction of pS2, pBRGDH and pGK into α -KGDH deficient strain Escherichia coli W3100 sucA::Km' and evaluation of L-glutamic acid-production

The α -KGDH-deficient strain, Escherichia coli W3100 sucA:: Km' was transformed with each of pS2, pBRGDH and pGK, and each of the transformed strains was inoculated into a 500-ml shaker flask containing 20 ml of the culture medium having the composition shown in Table 2. Cultivation was then carried out at 37 °C until the glucose in the culture medium was consumed completely. The results are shown in Table 3.

Table 2

	Component	Concentration (g/l)
35	Glucose	40
	(NH ₄) ₂ SO ₄	20
40	KH ₂ PO ₄	1
	MgSO ₄ ·7H ₂ O	1
	FeSO ₄ ·7H ₂ O	0.01
45	MnSO ₄ ·5H ₂ O	0.01
	Yeast extract	2
	Thiamine hydrochloride	0.01
50	CaCO ₃	50

Table 3

Strain	Accumulated L-glutamic acid (g/l)
W3110 sucA::Kmr	19.2
W3110 sucA::Kmr/pS2	19.9
W3110 sucA::Kmr/pBRGDH	2.8
W3110 sucA::Kmr/pGK (AJ 12949)	23.3

Although the transformed strain having the PPC activity amplified by the introduction of pS2 exhibited slightly reduced growth as compared with the host strain, W3110 sucA::Kmr, it accumulated L-glutamic acid in an amount similar to that accumulated by the host strain. The strain having GDH activity amplified by the introduction of pBRGDH exhibited quite poor growth, and the amount of the accumulated L-glutamic acid was surprisingly smaller than that accumulated by the strain W3110 sucA::Kmr.

On the contrary, the transformed strain in which both of PPC and GDH activities were amplified simultaneously by the introduction of pGK exhibited growth similar to that of the host strain while producing an increased amount of accumulated L-glutamic acid. *Escherichia coli* W3110 sucA::Kmr into which pGK plasmid having the ppc and gdhA genes had been introduced was designated as AJ 12949. *Escherichia coli* AJ 12949 was originally deposited under the accession number FERM P-14039 on December 28, 1993, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken 305, Japan, and the deposit was converted into a deposit under the Budapest Treaty under the accession number FERM BP-4881 on November 11, 1994.

The host strain, namely, W3110 sucA::Kmr can be obtained by curing the plasmid from the deposited strain, AJ 12949 without damaging the cell. The plasmid may be lost from AJ 12949 spontaneously, or may be cured in a curing procedure (Bact. Rev., Vol. 36, p.361 to 405 (1972)). An example of the curing procedure is as follows. The strain AJ 12949 is inoculated to the L-broth medium (Bactotrypton 10 g/l, yeast extract 5 g/l, NaCl 5 g/l, pH 7.2), and cultivated at 40°C overnight. The culture broth is diluted appropriately, and spread onto the L-medium. After incubating it at 37° C overnight, the colonies formed are transferred to the L-medium containing 100 µg/ml of ampicillin and then ampicillin-sensitive colonies are isolated. The strain thus obtained is W3110 sucA::Kmr.

Advantages of the Invention

The method according to the present invention provides a mutant of the genus *Escherichia* having a higher productivity of L-glutamic acid as well as the efficient and low-cost method for the production of L-glutamic acid.

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45

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55

SEQUENCE LISTING

5

GENERAL INFORMATION:

APPLICANT:

NAME: Ajinomoto Co., Inc.
10 STREET: 15-1, Kyobashi 1-chome
CITY: Chuo-ku, Tokyo
COUNTRY: Japan
POSTAL CODE: none

15 TITLE OF INVENTION: Method of producing L-glutamic acid by fermentation

NUMER OF SEQUENCES: 8

COMPUTER READABLE FORM:

20 MEDIUM TYPE: Diskette
COMPUTER: IBM PC compatible
OPERATING SYSTEM: MS-DOS

25

SEQUENCE DESCRIPTION:

SEQ ID No.: 1
Length : 21 base pairs
30 Type : Nucleotide
Strandedness : Single
Topology : Linear
Molecule type: Synthetic DNA
Feature : Primer for amplification of sucA gene of Escherichia coli
Sequence
35 ACGCGCAAGC GTCGCATCAG G

21

40 SEQ ID No.: 2
Length : 21 base pairs
Type : Nucleotide
Strandedness : Single
Topology : Linear
Molecule type: Synthetic DNA
45 Feature : Primer for amplification of sucA gene of Escherichia coli
Sequence
ATCGGCTACG AATTCAAGGCA G

21

50 SEQ ID No.: 3
Length : 20 base pairs
Type : Nucleotide
Strandedness : Single
Topology : Linear
55 Molecule type: Synthetic DNA
Feature : Primer for amplification of sucB gene of Escherichia coli

Sequence
 5 CCGGTCGCGG TACCTTCTTC 20

SEQ ID No.: 4
 Length : 26 base pairs
 Type : Nucleotide
 Strandedness : Single
 Topology : Linear
 Molecule type: Synthetic DNA
 Feature : Primer for amplification of sucB gene of Escherichia coli
 15 Sequence CGTAGACCGA ATTCTTCGTA TCGCTT 26

SQ ID No.: 5
 Length : 21 base pairs
 Type : Nucleotide
 Strandedness : Single
 Topology : Linear
 Molecule type: Synthetic DNA
 Feature : Primer for amplification of gdhA gene of Escherichia coli
 25 Sequence GGGTGGCAAA GCTTTAGCGT C 21

SEQ ID No.: 6
 Length : 21 base pairs
 Type : Nucleotide
 Strandedness : Single
 Topology : Linear
 Molecule type: Synthetic DNA
 Feature : Primer for amplification of gdhA gene of Escherichia coli
 35 Sequence TCGAGAACCA TGCATTATAT A 21

SEQ ID No.: 7
 Length : 4623 base pairs
 Type : Nucleotide
 Strandedness : Single
 Topology : Linear
 Molecule type: Genomic DNA
 Original source
 Organism : Escherichia coli
 Features
 45 Feature key : CDS ⇒from 327 to 318 bp coding sequence
 Location : 327..3128
 Method of feature determination : E
 Feature key : CDS ⇒from 3143 to 4357 bp coding sequence
 Location : 3143..4357
 Method of feature determination : E
 50
 55

Sequence		
5	TCGATGTTGT TGCAACGTAA TGCGTAAACC GTAGGCCTGA TAAGACGCGC AAGCGTCGCA	60
	TCAGGCCAAC AGTGCCTGGAT GCGCGTGAAC GCCTTATCCG GCCTACAAGT CATTACCCGT	120
	AGGCCCTGATA AGCGCAGCGC ATCAGCGTA ACAAAAGAAAT GCAGGAAATC TTAAAAAAACT	180
	GCCCCCTGACA CTAAGACAGT TTTTAAAGGT TCCTTCGCGA GCCACTACGT AGACAAGAGC	240
	TCGCAAGTGA ACCCCGGCAC GCACATCACT GTGCGTGGTA GTATCCACGG CGAAGTAAGC	300
	ATAAAAAAAAGA TGCTTAAGGG ATCACG ATG CAG AAC AGC GCT TTG AAA GCC TGG	353
	Met Gln Asn Ser Ala Leu Lys Ala Trp	
10	1 5	
	TTG GAC TCT TCT TAC CTC TCT GGC GCA AAC CAG AGC TGG ATA GAA CAG	401
	Leu Asp Ser Ser Tyr Leu Ser Gly Ala Asn Gln Ser Trp Ile Glu Gln	
	10 15 20 25	
	CTC TAT GAA GAC TTC TTA ACC GAT CCT GAC TCG GTT GAC GCT AAC TGG	449
	Leu Tyr Glu Asp Phe Leu Thr Asp Pro Asp Ser Val Asp Ala Asn Trp	
15	30 35 40	
	CGT TCG ACG TTC CAG CAG TTA CCT GGT ACG GGA GTC AAA CCG GAT CAA	497
	Arg Ser Thr Phe Gln Gln Leu Pro Gly Thr Gly Val Lys Pro Asp Gln	
	45 50 55	
	TTC CAC TCT CAA ACG CGT GAA TAT TTC CGC CGC CTG GCG AAA GAC GCT	545
	Phe His Ser Gln Thr Arg Glu Tyr Phe Arg Arg Leu Ala Lys Asp Ala	
20	60 65 70	
	TCA CGT TAC TCT TCA ACG ATC TCC GAC CCT GAC ACC AAT GTG AAG CAG	593
	Ser Arg Tyr Ser Ser Thr Ile Ser Asp Pro Asp Thr Asn Val Lys Gln	
	75 80 85	
	GTT AAA GTC CTG CAG CTC ATT AAC GCA TAC CGC TTC CGT GGT CAC CAG	641
	Val Lys Val Leu Gln Leu Ile Asn Ala Tyr Arg Phe Arg Gly His Gln	
25	90 95 100 105	
	CAT GCG AAT CTC GAT CCG CTG GGA CTG TGG CAG CAA GAT AAA GTG GCC	689
	His Ala Asn Leu Asp Pro Leu Gly Leu Trp Gln Gln Asp Lys Val Ala	
	110 115 120	
	GAT CTG GAT CCG TCT TTC CAC GAT CTG ACC GAA GCA GAC TTC CAG GAG	737
	Asp Leu Asp Pro Ser Phe His Asp Leu Thr Glu Ala Asp Phe Gln Glu	
30	125 130 135	
	ACC TTC AAC GTC GGT TCA TTT GCC AGC GGC AAA GAA ACC ATG AAA CTC	785
	Thr Phe Asn Val Gly Ser Phe Ala Ser Gly Lys Glu Thr Met Lys Leu	
	140 145 150	
	GGC GAG CTG CTG GAA GCC CTC AAG CAA ACC TAC TGC GGC CCG ATT GGT	833
	Gly Glu Leu Leu Glu Ala Leu Lys Gln Thr Tyr Cys Gly Pro Ile Gly	
35	155 160 165	
	GCC GAG TAT ATG CAC ATT ACC AGC ACC GAA GAA AAA CGC TGG ATC CAA	881
	Ala Glu Tyr Met His Ile Thr Ser Thr Glu Glu Lys Arg Trp Ile Gln	
	170 175 180 185	
	CAG CGT ATC GAG TCT GGT CGC GCG ACT TTC AAT AGC GAA GAG AAA AAA	929
	Gln Arg Ile Glu Ser Gly Arg Ala Thr Phe Asn Ser Glu Glu Lys Lys	
40	190 195 200	
	CGC TTC TTA AGC GAA CTG ACC GCC GCT GAA GGT CTT GAA CGT TAC CTC	977
	Arg Phe Leu Ser Glu Leu Thr Ala Ala Glu Gly Leu Glu Arg Tyr Leu	
	205 210 215	
	GGC GCA AAA TTC CCT GGC GCA AAA CGC TTC TCG CTG GAA GGC GGT GAC	1025
	Gly Ala Lys Phe Pro Gly Ala Lys Arg Phe Ser Leu Glu Gly Gly Asp	
45	220 225 230	
	GCG TTA ATC CCG ATG CTT AAA GAG ATG ATC CGC CAC GCT GGC AAC AGC	1073
	Ala Leu Ile Pro Met Leu Lys Glu Met Ile Arg His Ala Gly Asn Ser	
	235 240 245	
	GGC ACC CGC GAA GTG GTT CTC GGG ATG GCG CAC CGT GGT CGT CTG AAC	1121
	Gly Thr Arg Glu Val Val Leu Gly Met Ala His Arg Gly Arg Leu Asn	
50	250 255 260	
	GTG CTG GTG AAC GTG CTG GGT AAA AAA CCG CAA GAC TTG TTC GAC GAG	1169
	Val Leu Val Asn Val Leu Gly Lys Lys Pro Gln Asp Leu Phe Asp Glu	
	270 275 280	
	TTC GCC GGT AAA CAT AAA GAA CAC CTC GGC ACG GGT GAC GTG AAA TAC	1217
55	Phe Ala Gly Lys His Lys Glu His Leu Gly Thr Gly Asp Val Lys Tyr	

	285	290	295	
5	CAC ATG GGC TTC TCG TCT GAC TTC CAG ACC GAT GGC GGC CTG GTG CAC His Met Gly Phe Ser Ser Asp Phe Gln Thr Asp Gly Gly Leu Val His			1265
	300	305	310	
	CTG GCG CTG GCG TTT AAC CCG TCT CAC CTT GAG ATT GTA AGC CCG GTA Leu Ala Leu Ala Phe Asn Pro Ser His Leu Glu Ile Val Ser Pro Val			1313
	315	320	325	
10	GTT ATC GGT TCT GTT CGT GCC CGT CTG GAC AGA CTT GAT GAG CCG AGC Val Ile Gly Ser Val Arg Ala Arg Leu Asp Arg Leu Asp Glu Pro Ser			1361
	330	335	340	345
	AGC AAC AAA GTG CTG CCA ATC ACC ATC CAC GGT GAC GCC GCA GTG ACC Ser Asn Lys Val Leu Pro Ile Thr Ile His Gly Asp Ala Ala Val Thr			1409
	350	355	360	
15	GGG CAG GGC GTG GTT CAG GAA ACC CTG AAC ATG TCG AAA GCG CGT GGT Gly Gln Gly Val Val Gln Glu Thr Leu Asn Met Ser Lys Ala Arg Gly			1457
	365	370	375	
	TAT GAA GTT GGC GGT ACG GTA CGT ATC GTT ATC AAC AAC CAG GTT GGT Tyr Glu Val Gly Gly Thr Val Arg Ile Val Ile Asn Asn Gln Val Gly			1505
	380	385	390	
20	TTC ACC ACC TCT AAT CCG CTG GAT GCC CGT TCT ACG CCG TAC TGT ACT Phe Thr Thr Ser Asn Pro Leu Asp Ala Arg Ser Thr Pro Tyr Cys Thr			1553
	395	400	405	
	GAT ATC GGT AAG ATG GTT CAG GCC CCG ATT TTC CAC GTT AAC GCG GAC Asp Ile Gly Lys Met Val Gln Ala Pro Ile Phe His Val Asn Ala Asp			1601
	410	415	420	425
25	GAT CCG GAA GCC GTT GCC TTT GTG ACC CGT CTG GCG CTC GAT TTC CGT Asp Pro Glu Ala Val Ala Phe Val Thr Arg Leu Ala Leu Asp Phe Arg			1649
	430	435	440	
	AAC ACC TTT AAA CGT GAT GTC TTC ATC GAC CTG GTG TCG TAC CGC CGT Asn Thr Phe Lys Arg Asp Val Phe Ile Asp Leu Val Ser Tyr Arg Arg			1697
	445	450	455	
30	CAC GGC CAC AAC GAA GCC GAC GAG CCG AGC GCA ACC CAG CCG CTG ATG His Gly His Asn Glu Ala Asp Glu Pro Ser Ala Thr Gln Pro Leu Met			1745
	460	465	470	
	TAT CAG AAA ATC AAA AAA CAT CCG ACA CCG CGC AAA ATC TAC GCT GAC Tyr Gln Lys Ile Lys His Pro Thr Pro Arg Lys Ile Tyr Ala Asp			1793
	475	480	485	
35	AAG CTG GAG CAG GAA AAA GTG GCG ACG CTG GAA GAT GCC ACC GAG ATG Lys Leu Glu Gln Glu Lys Val Ala Thr Leu Glu Asp Ala Thr Glu Met			1841
	490	495	500	505
	GTT AAC CTG TAC CGC GAT GCG CTG GAT GCT GGC GAT TGC GTA GTG GCA Val Asn Leu Tyr Asp Ala Leu Asp Ala Gly Asp Cys Val Val Ala			1889
	510	515	520	
40	GAG TGG CGT CCG ATG AAC ATG CAC TCT TTC ACC TGG TCG CCG TAC CTC Glu Trp Arg Pro Met Asn Met His Ser Phe Thr Trp Ser Pro Tyr Leu			1937
	525	530	535	
	AAC CAC GAA TGG GAC GAA GAG TAC CCG AAC AAA GTT GAG ATG AAG CGC Asn His Glu Trp Asp Glu Glu Tyr Pro Asn Lys Val Glu Met Lys Arg			1985
	540	545	550	
45	CTG CAG GAG CTG GCG AAA CGC ATC AGC ACG GTG CCG GAA GCA GTT GAA Leu Gln Glu Leu Ala Lys Arg Ile Ser Thr Val Pro Glu Ala Val Glu			2033
	555	560	565	
	ATG CAG TCT CGC GTT GCC AAG ATT TAT GGC GAT CGC CAG GCG ATG GCT Met Gln Ser Arg Val Ala Lys Ile Tyr Gly Asp Arg Gln Ala Met Ala			2081
	570	575	580	585
50	GCC GGT GAG AAA CTG TTC GAC TGG GGC GGT GCG GAA AAC CTC GCT TAC Ala Gly Glu Lys Leu Phe Asp Trp Gly Gly Ala Glu Asn Leu Ala Tyr			2129
	590	595	600	
	GCC ACG CTG GTT GAT GAA GGC ATT CCG GTT CGC CTG TCG GGT GAA GAC Ala Thr Leu Val Asp Glu Gly Ile Pro Val Arg Leu Ser Gly Glu Asp			2177
	605	610	615	
55	TCC GGT CGC GGT ACC TTC TTC CAC CGC CAC GCG GTG ATC CAC AAC CAG			2225

5	Ser Gly Arg Gly Thr Phe Phe His Arg His Ala Val Ile His Asn Gln 620 625 630	
	TCT AAC GGT TCC ACT TAC ACG CCG CTG CAA CAT ATC CAT AAC GGG CAG Ser Asn Gly Ser Thr Tyr Thr Pro Leu Gln His Ile His Asn Gly Gln 635 640 645	2273
10	Gly GCG TTC CGT GTC TGG GAC TCC GTA CTG TCT GAA GAA GCA GTG CTG Gly Ala Phe Arg Val Trp Asp Ser Val Leu Ser Glu Glu Ala Val Leu 650 655 660 665	2321
	GCG TTT GAA TAT GGT TAT GCC ACC GCA GAA CCA CGC ACT CTG ACC ATC Ala Phe Glu Tyr Gly Tyr Ala Thr Ala Glu Pro Arg Thr Leu Thr Ile 670 675 680	2369
15	TGG GAA GCG CAG TTC GGT GAC TTC GCC AAC GGT GCG CAG GTG GTT ATC Trp Glu Ala Gln Phe Gly Asp Phe Ala Asn Gly Ala Gln Val Val Ile 685 690 695	2417
	GAC CAG TTC ATC TCC TCT GGC GAA CAG AAA TGG GGC CGG ATG TGT GGT Asp Gln Phe Ile Ser Ser Gly Glu Gln Lys Trp Gly Arg Met Cys Gly 700 705 710	2465
20	CTG GTG ATG TTG CTG CCG CAC GGT TAC GAA GGG CAG GGG CCG GAG CAC Leu Val Met Leu Leu Pro His Gly Tyr Glu Gly Gln Gly Pro Glu His 715 720 725	2513
	TCC TCC GCG CGT CTG GAA CGT TAT CTG CAA CTT TGT GCT GAG CAA AAC Ser Ser Ala Arg Leu Glu Arg Tyr Leu Gln Leu Cys Ala Glu Gln Asn 730 735 740 745	2561
25	ATG CAG GTT TGC GTA CCG TCT ACC CCG GCA CAG GTT TAC CAC ATG CTG Met Gln Val Cys Val Pro Ser Thr Pro Ala Gln Val Tyr His Met Leu 750 755 760	2609
	CGT CGT CAG GCG CTG CGC GGG ATG CGT CGT CCG CTG GTC GTG ATG TCG Arg Arg Gln Ala Leu Arg Gly Met Arg Arg Pro Leu Val Val Met Ser 765 770 775	2657
30	CCG AAA TCC CTG CTG CGT CAT CCG CTG GCG GTT TCC AGC CTC GAA GAA Pro Lys Ser Leu Leu Arg His Pro Leu Ala Val Ser Ser Leu Glu Glu 780 785 790	2705
	CTG GCG AAC GGC ACC TTC CTG CCA GCC ATC GGT GAA ATC GAC GAG CTT Leu Ala Asn Gly Thr Phe Leu Pro Ala Ile Gly Glu Ile Asp Glu Leu 795 800 805	2753
35	GAT CCG AAG GGC GTG AAG CGC GTA GTG ATG TGT TCT GGT AAG GTT TAT Asp Pro Lys Gly Val Lys Arg Val Val Met Cys Ser Gly Lys Val Tyr 810 815 820 825	2801
	TAC GAC CTG CTG GAA CAG CGT CGT AAG AAC AAT CAA CAC GAT GTC GCC Tyr Asp Leu Leu Glu Gln Arg Arg Lys Asn Asn Gln His Asp Val Ala 830 835 840	2849
40	ATT GTG CGT ATC GAG CAA CTC TAC CCG TTC CCG CAT AAA GCG ATG CAG Ile Val Arg Ile Glu Gln Leu Tyr Pro Phe Pro His Lys Ala Met Gln 845 850 855	2897
	GAA GTG TTG CAG CAG TTT GCT CAC GTC AAG GAT TTT GTC TGG TGC CAG Glu Val Leu Gln Phe Ala His Val Lys Asp Phe Val Trp Cys Gln 860 865 870	2945
45	GAA GAG CCG CTC AAC CAG GGC GCA TGG TAC TGC AGC CAG CAT CAT TTC Glu Glu Pro Leu Asn Gln Gly Ala Trp Tyr Cys Ser Gln His His Phe 875 880 885	2993
	CGT GAA GTG ATT CCG TTT GGG GCT TCT CTG CGT TAT GCA GGC CGC CCG Arg Glu Val Ile Pro Phe Gly Ala Ser Leu Arg Tyr Ala Gly Arg Pro 890 895 900 905	3041
50	GCC TCC GCC TCT CCG GCG GTA GGG TAT ATG TCC GTT CAC CAG AAA CAG Ala Ser Ala Ser Pro Ala Val Gly Tyr Met Ser Val His Gln Lys Gln 910 915 920	3089
	CAA CAA GAT CTG GTT AAT GAC GCG CTG AAC GTC GAA TAAATAAAGG Gln Gln Asp Leu Val Asn Asp Ala Leu Asn Val Glu 925 930	3135
55	ATACACA ATG AGT AGC GTA GAT ATT CTG GTC CCT GAC CTG CCT GAA TCC Met Ser Ser Val Asp Ile Leu Val Pro Asp Leu Pro Glu Ser	3184

	GTA GCC GAT GCC ACC GTC GCA ACC TGG CAT AAA AAA CCC GGC GAC GCA	3232
5	Val Ala Asp Ala Thr Val Ala Thr Trp His Lys Lys Pro Gly Asp Ala 15 20 25 30	
	GTC GTA CGT GAT GAA GTG CTG GTA GAA ATC GAA ACT GAC AAA GTG GTA	3280
	Val Val Arg Asp Glu Val Leu Val Glu Ile Glu Thr Asp Lys Val Val 35 40 45	
10	CTG GAA GTA CCG GCA TCA GCA GAC GGC ATT CTG GAT GCG GTT CTG GAA	3328
	Leu Glu Val Pro Ala Ser Ala Asp Gly Ile Leu Asp Ala Val Leu Glu 50 55 60	
	GAT GAA GGT ACA ACG GTA ACG TCT CGT CAG ATC CTT GGT CGC CTG CGT	3376
	Asp Glu Gly Thr Thr Val Thr Ser Arg Gln Ile Leu Gly Arg Leu Arg 65 70 75	
15	GAA GGC AAC AGC GCC GGT AAA GAA ACC AGC GCC AAA TCT GAA GAG AAA	3424
	Glu Gly Asn Ser Ala Gly Lys Glu Thr Ser Ala Lys Ser Glu Glu Lys 80 85 90	
	GCG TCC ACT CCG GCG CAA CGC CAG CAG GCG TCT CTG GAA GAG CAA AAC	3472
	Ala Ser Thr Pro Ala Gln Arg Gln Gln Ala Ser Leu Glu Glu Gln Asn 95 100 105 110	
20	AAC GAT GCG TTA AGC CCG GCG ATC CGT CGC CTG CTG GCT GAA CAC AAT	3520
	Asn Asp Ala Leu Ser Pro Ala Ile Arg Arg Leu Leu Ala Glu His Asn 115 120 125	
	CTC GAC GCC AGC GCC ATT AAA GGC ACC GGT GTG GGT GGT CGT CTG ACT	3568
	Leu Asp Ala Ser Ala Ile Lys Gly Thr Val Gly Gly Arg Leu Thr 130 135 140	
25	CGT GAA GAT GTG GAA AAA CAT CTG GCG AAA GCC CCG GCG AAA GAG TCT	3616
	Arg Glu Asp Val Glu Lys His Leu Ala Lys Ala Pro Ala Lys Glu Ser 145 150 155	
	GCT CCG GCA GCG GCT CCG GCG CAA CCG GCT CTG GCT GCA CGT	3664
	Ala Pro Ala Ala Ala Pro Ala Ala Gln Pro Ala Leu Ala Ala Arg 160 165 170	
30	AGT GAA AAA CGT GTC CCG ATG ACT CGC CTG CGT AAG CGT GTG GCA GAG	3712
	Ser Glu Lys Arg Val Pro Met Thr Arg Leu Arg Lys Arg Val Ala Glu 175 180 185 190	
	CGT CTG CTG GAA GCG AAA AAC TCC ACC GCC ATG CTG ACC ACG TTC AAC	3760
	Arg Leu Leu Glu Ala Lys Asn Ser Thr Ala Met Leu Thr Thr Phe Asn 195 200 205	
35	GAA GTC AAC ATG AAG CCG ATT ATG GAT CTG CGT AAG CAG TAC GGT GAA	3808
	Glu Val Asn Met Lys Pro Ile Met Asp Leu Arg Lys Gln Tyr Gly Glu 210 215 220	
	GCG TTT GAA AAA CGC CAC GGC ATC CGT CTG GGC TTT ATG TCC TTC TAC	3856
	Ala Phe Glu Lys Arg His Gly Ile Arg Leu Gly Phe Met Ser Phe Tyr 225 230 235	
40	GTG AAA GCG GTG GTT GAA GCC CTG AAA CGT TAC CCG GAA GTG AAC GCT	3904
	Val Lys Ala Val Val Glu Ala Leu Lys Arg Tyr Pro Glu Val Asn Ala 240 245 250	
	TCT ATC GAC GGC GAT GAC GTG GTT TAC CAC AAC TAT TTC GAC GTC AGC	3952
	Ser Ile Asp Gly Asp Asp Val Val Tyr His Asn Tyr Phe Asp Val Ser 255 260 265 270	
45	ATG GCG GTT TCT ACG CCG CGC GGC CTG GTG ACG CCG GTT CTG CGT GAT	4000
	Met Ala Val Ser Thr Pro Arg Gly Leu Val Thr Pro Val Leu Arg Asp 275 280 285	
	GTC GAT ACC CTC GGC ATG GCA GAC ATC GAG AAG AAA ATC AAA GAG CTG	4048
	Val Asp Thr Leu Gly Met Ala Asp Ile Glu Lys Lys Ile Lys Glu Leu 290 295 300	
50	GCA GTC AAA GGC CGT GAC GGC AAG CTG ACC GTT GAA GAT CTG ACC GGT	4096
	Ala Val Lys Gly Arg Asp Gly Lys Leu Thr Val Glu Asp Leu Thr Gly 305 310 315	
	GGT AAC TTC ACC ATC ACC AAC GGT GGT GTG TTC GGT TCC CTG ATG TCT	4144
	Gly Asn Phe Thr Ile Thr Asn Gly Gly Val Phe Gly Ser Leu Met Ser 320 325 330	
55	ACG CCG ATC ATC AAC CCG CCG CAG AGC GCA ATT CTG GGT ATG CAC GCT	4192
	Thr Pro Ile Ile Asn Pro Pro Gln Ser Ala Ile Leu Gly Met His Ala	

5	335	340	345	350	
	ATC AAA GAT CGT CCG ATG GCG GTG AAT GGT	CAG GTT GAG ATC CTG CCG			4240
	Ile Lys Asp Arg Pro Met Ala Val Asn Gly	Gln Val Glu Ile Leu Pro			
	355	360	365		
	ATG ATG TAC CTG GCG CTG TCC TAC GAT CAC CGT CTG ATC GAT GGT CGC				4288
	Met Met Tyr Leu Ala Leu Ser Tyr Asp His Arg Leu Ile Asp Gly Arg				
10	370	375	380		
	GAA TCC GTG GGC TTC CTG GTA ACG ATC AAA GAG TTG CTG GAA GAT CCG				4336
	Glu Ser Val Gly Phe Leu Val Thr Ile Lys Glu Leu Leu Glu Asp Pro				
	385	390	395		
	ACG CGT CTG CTG GAC GTG TAGTAGTTA AGTTTCACCT GCACGTAGA				4387
	Thr Arg Leu Leu Asp Val				
	400	405			
15	CCGGATAAGG CATTATCGCC TTCTCCGCC ATTGAAGCCT GATGCGACGC TGACGCGTCT				4447
	TATCAGGCCT ACGGGACCAC CAATGTAGGT CGGATAAGGC GCAACGCCGC ATCCGACAAG				4507
	CGATGCCCTGA TGTGACGTT AACGTGTCTT ATCAGGCCCTA CGGGTGACCG ACAATGCCCG				4567
	GAAGCGATAC GAAAATTCG GTCTACGGTT TAAAAGATAA CGATTACTGA AGGATG				4623
20	SEQ ID No.: 8				
	Length : 1937 base pairs				
	Type : Nucleotide				
	Strandedness : Single				
	Topology : Linear				
25	Molecule type: Genomic DNA				
	Original source				
	Organism : <u>Escherichia coli</u>				
	Sequence feature				
	Feature key : CDS	⇒from 194 to 1537 bp coding sequence			
	Location : 194..1537				
30	Method of feature determination : E				
	Sequence				
	CCGGGTGGCA AAACTTTAGC GTCTGAGGTT ATCGCAATT GGTTATGAGA TTACTCTCGT				60
	TATTAATTTG CTTTCCTGGG TCATTTTTT CTTGCTTACC GTCACATTCT TGATGGTATA				120
	GTCGAAACT GCAAAGCAC ATGACATAAA CAACATAAGC ACAATCGTAT TAATATATAAA				180
	GGGTTTTATA TCT ATG GAT CAG ACA TAT TCT CTG GAG TCA TTC CTC AAC				229
35	Met Asp Gln Thr Tyr Ser Leu Glu Ser Phe Leu Asn				
	1 5 10				
	CAT GTC CAA AAG CGC GAC CCG AAT CAA ACC GAG TTC GCG CAA GCC GTT				
	His Val Gln Lys Arg Asp Pro Asn Gln Thr Glu Phe Ala Gln Ala Val				
	15 20 25				
40	CGT GAA GTA ATG ACC ACA CTC TGG CCT TTT CTT GAA CAA AAT CCA AAA				325
	Arg Glu Val Met Thr Leu Trp Pro Phe Leu Glu Gln Asn Pro Lys				
	30 35 40				
	TAT CGC CAG ATG TCA TTA CTG GAG CGT CTG GTT GAA CCG GAG CGC GTG				373
	Tyr Arg Gln Met Ser Leu Leu Glu Arg Leu Val Glu Pro Glu Arg Val				
	45 50 55 60				
45	ATC CAG TTT CGC GTG GTA TGG GTT GAT GAT CGC AAC CAG ATA CAG GTC				421
	Ile Gln Phe Arg Val Val Trp Val Asp Asp Arg Asn Gln Ile Gln Val				
	65 70 75				
	AAC CGT GCA TGG CGT GTG CAG TTC AGC TCT GCC ATC GGC CCG TAC AAA				469
	Asn Arg Ala Trp Arg Val Gln Phe Ser Ser Ala Ile Gly Pro Tyr Lys				
	80 85 90				
50	GGC GGT ATG CGC TTC CAT CCG TCA GTT AAC CTT TCC ATT CTC AAA TTC				517
	Gly Gly Met Arg Phe His Pro Ser Val Asn Leu Ser Ile Leu Lys Phe				
	95 100 105				
	CTC GGC TTT GAA CAA ACC TTC AAA AAT GCC CTG ACT ACT CTG CCG ATG				565
	Leu Gly Phe Glu Gln Thr Phe Lys Asn Ala Leu Thr Thr Leu Pro Met				
	110 115 120				
55	GGC GGT GGT AAA GGC GGC AGC GAT TTC GAT CCG AAA GGA AAA AGC GAA				613
	Gly Gly Gly Lys Gly Ser Asp Phe Asp Pro Lys Gly Lys Ser Glu				

	125	130	135	140	
5	GGT GAA GTG ATG CGT TTT TGC CAG GCG CTG ATG ACT GAA CTG TAT CGC Gly Glu Val Met Arg Phe Cys Gln Ala Leu Met Thr Glu Leu Tyr Arg		145	150	155
					661
	CAC CTG GGC GCG GAT ACC GAC GTT CCG GCA GGT GAT ATC GGG GTT GGT His Leu Gly Ala Asp Thr Asp Val Pro Ala Gly Asp Ile Gly Val Gly		160	165	170
10					709
	GGT CGT GAA GTC GGC TTT ATG GCG GGG ATG ATG AAA AAG CTC TCC AAC Gly Arg Glu Val Gly Phe Met Ala Gly Met Met Lys Lys Leu Ser Asn		175	180	185
					757
	AAT ACC GCC TGC GTC TTC ACC GGT AAG GGC CTT TCA TTT GGC GGC AGT Asn Thr Ala Cys Val Phe Thr Gly Lys Gly Leu Ser Phe Gly Gly Ser		190	195	200
15					805
	CTT ATT CGC CCG GAA GCT ACC GGC TAC GGT CTG GTT TAT TTC ACA GAA Leu Ile Arg Pro Glu Ala Thr Gly Tyr Gly Leu Val Tyr Phe Thr Glu		205	210	215
					853
	GCA ATG CTA AAA CGC CAC GGT ATG GGT TTT GAA GGG ATG CGC GTT TCC Ala Met Leu Lys Arg His Gly Met Gly Phe Glu Gly Met Arg Val Ser		225	230	235
20					901
	GTT TCT GGC TCC GGC AAC GTC GCC CAG TAC GCT ATC GAA AAA GCG ATG Val Ser Gly Ser Gly Asn Val Ala Gln Tyr Ala Ile Glu Lys Ala Met		240	245	250
					949
	GAA TTT GGT GCT CGT GTG ATC ACT GCG TCA GAC TCC AGC GGC ACT GTA Glu Phe Gly Ala Arg Val Ile Thr Ala Ser Asp Ser Ser Gly Thr Val		255	260	265
25					997
	GTT GAT GAA AGC GGA TTC ACG AAA GAG AAA CTG GCA CGT CTT ATC GAA Val Asp Glu Ser Gly Phe Thr Lys Glu Lys Leu Ala Arg Leu Ile Glu		270	275	280
					1045
	ATC AAA GCC AGC CGC GAT GGT CGA GTG GCA GAT TAC GCC AAA GAA TTT Ile Lys Ala Ser Arg Asp Gly Arg Val Ala Asp Tyr Ala Lys Glu Phe		285	290	295
30					1093
	295	300			
	GGT CTG GTC TAT CTC GAA GGC CAA CAG CCG TGG TCT CTA CCG GTT GAT Gly Leu Val Tyr Leu Glu Gly Gln Gln Pro Trp Ser Leu Pro Val Asp		305	310	315
					1141
	ATC GCC CTG CCT TGC GCC ACC CAG AAT GAA CTG GAT GTT GAC GCC GCG Ile Ala Leu Pro Cys Ala Thr Gln Asn Glu Leu Asp Val Asp Ala Ala		320	325	330
35					1189
	CAT CAG CTT ATC GCT AAT GGC GTT AAA GCC GTC GCC GAA GGG GCA AAT His Gln Leu Ile Ala Asn Gly Val Lys Ala Val Ala Glu Gly Ala Asn		335	340	345
					1237
	ATG CCG ACC ACC ATC GAA GCG ACT GAA CTG TTC CAG CAG GCA GGC GTA Met Pro Thr Thr Ile Glu Ala Thr Glu Leu Phe Gln Gln Ala Gly Val		350	355	360
40					1285
	360	365			
	CTA TTT GCA CCG GGT AAA GCG GCT AAT GCT GTT GGC GTC GCT ACA TCG Leu Phe Ala Pro Gly Lys Ala Ala Asn Ala Gly Val Ala Thr Ser		370	375	380
					1333
	380	385			
	GGC CTG GAA ATG CCA CAA AAC GCT GCG CGC CTG GGC TGG AAA GCC GAG Gly Leu Glu Met Pro Gln Asn Ala Ala Arg Leu Gly Trp Lys Ala Glu		390	395	
45					1381
	395	400			
	AAA GTT GAC GCA CGT TTG CAT CAC ATC ATG CTG GAT ATC CAC CAT GCC Lys Val Asp Ala Arg Leu His His Ile Met Leu Asp Ile His His Ala		405	410	
					1429
	410	415			
	TGT GTT GAG CAT GGT GGT GAA GGT GAG CAA ACC AAC TAC GTG CAG GGC Cys Val Glu His Gly Gly Glu Gln Thr Asn Tyr Val Gln Gly		420	425	
50					1477
	425	430			
	GCG AAC ATT GCC GGT TTT GTG AAG GTT GCC GAT GCG ATG CTG GCG CAG Ala Asn Ile Ala Gly Phe Val Lys Val Ala Asp Ala Met Leu Ala Gln		435	440	
					1525
	440	445			
	GGT GTG ATT TAAGTTGTAA ATGCCTGATG GCGCTACGCT TATCAGGCCT Gly Val Ile				1574
55	445	450			
	ACAAATGGGC ACAATTCAATT GCAGTTACGC TCTAATGTAG GCCGGGCAAG CGCAGCGCCC				1634

5 CCGGCAAAAT TTCAGGC GTT TATGAGT ATT TAACGGATGA TGCTCCCCAC GGAACATTT
TTATGGCCA ACGGCATTTC TTACTGTAGT GCTCCAAA CTGCTTGTCG TAACGATAAC
ACGCTTCAAG TTCAAGCATCC GTTAACTTTC TGCGGACTCA CGCGCCAGC ACTATGCCAG
TAAAGAAAATC CCATTTGACT ATTTTTTGA TAATCTTCTT CGCTTCGAA CAACTCGTGC
GCCTTCGAG AAGCAAGCAT TATATAATGC CAGGCCAGTT CTTCTTCAT TGCCCCGTTT
TGA

1694
1754
1814
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Claims

1. A mutant of the genus Escherichia having L-glutamic acid-productivity, said mutant having deficient or reduced α -ketoglutarate dehydrogenase activity and enhanced phosphoenolpyruvate carboxylase and glutamate dehydrogenase activities.
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2. A method of producing L-glutamic acid by fermentation comprising culturing in a liquid culture medium a mutant of the genus Escherichia having L-glutamic acid-productivity said mutant having deficient or reduced α -ketoglutarate dehydrogenase activity and enhanced phosphoenolpyruvate carboxylase and glutamate dehydrogenase activities, accumulating L-glutamic acid in the culture, and recovering L-glutamic acid therefrom.
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Fig.1

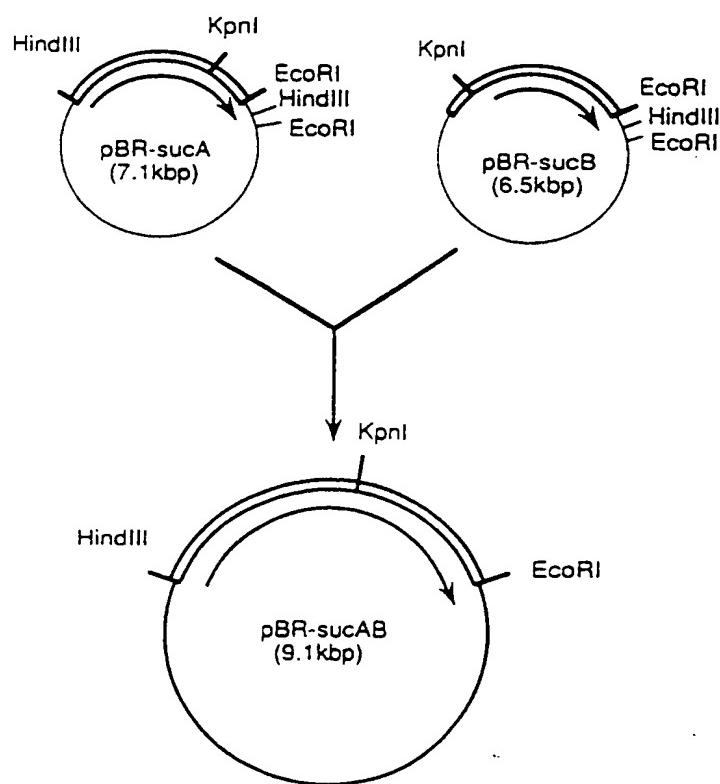


Fig. 2

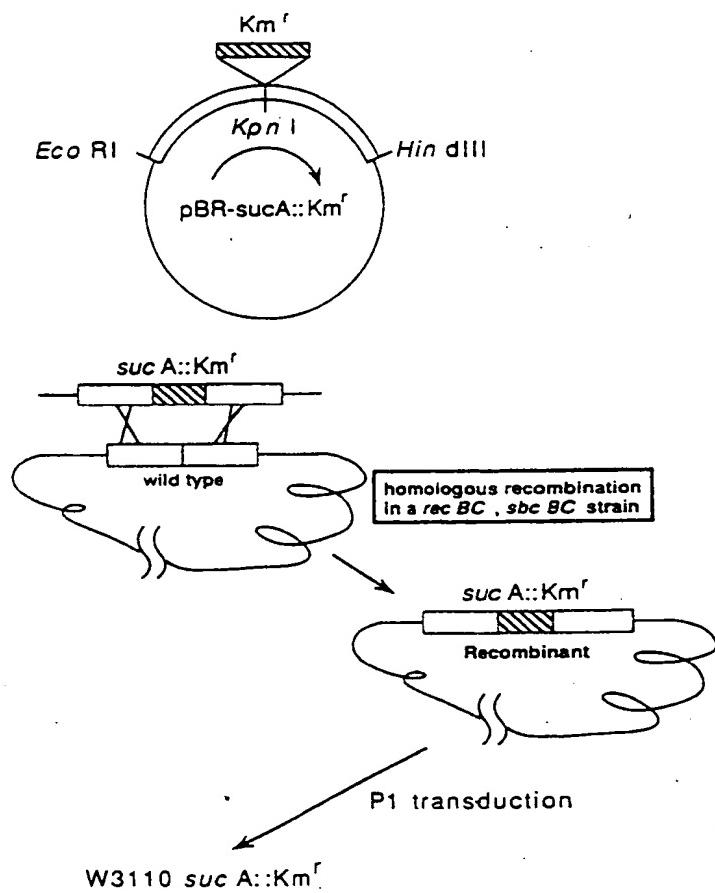
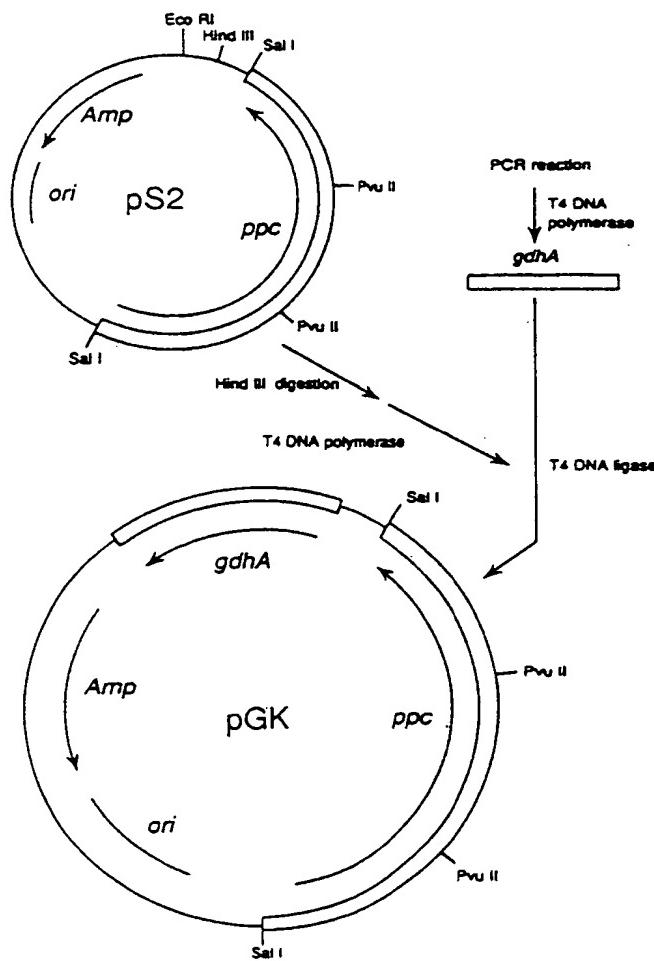
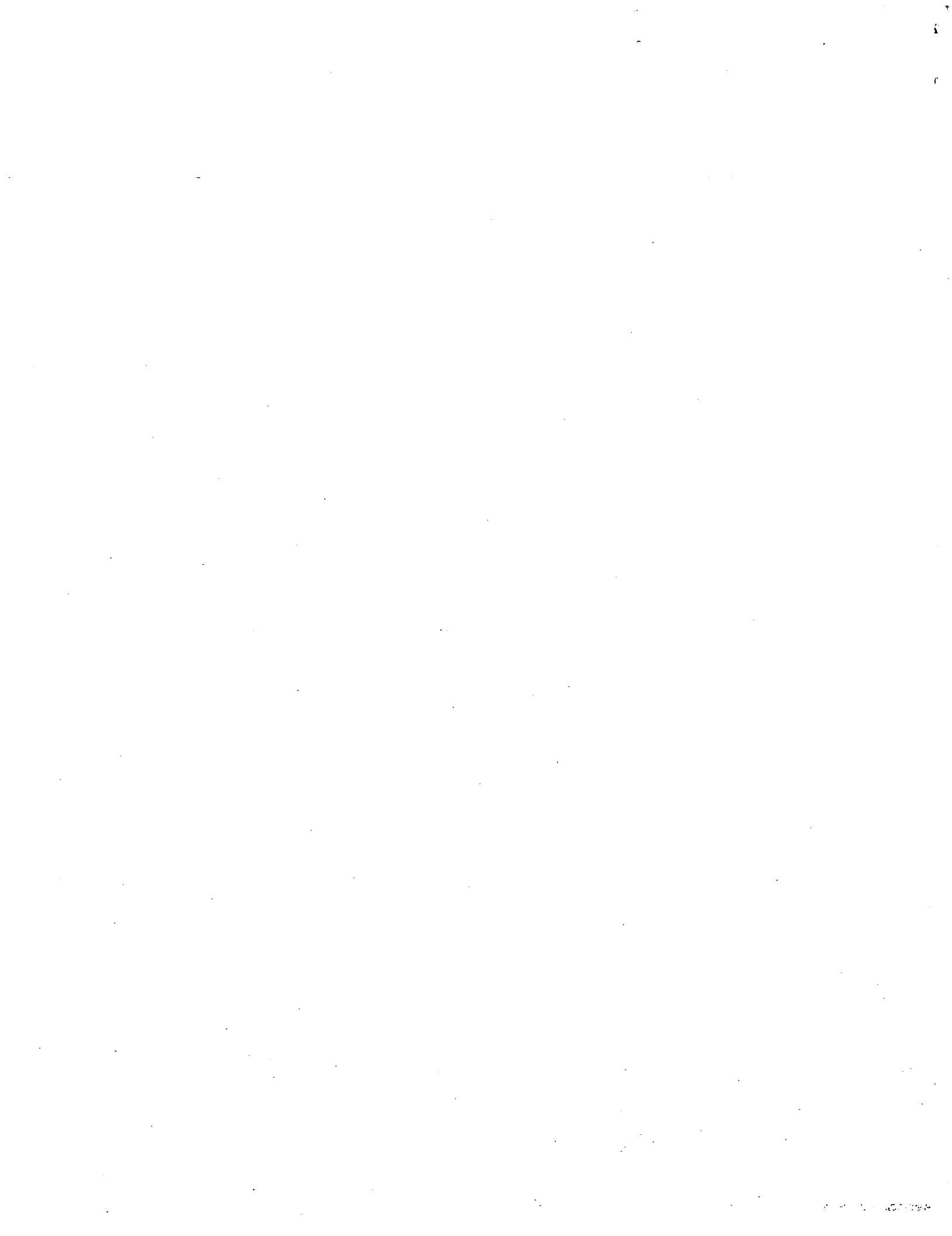


Fig. 3





(19)



Europäische Patentamt

European Patent Office

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(11)



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(12)

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(54) Method of producing L-glutamic acid by fermentation

(57) The present invention provides a low-cost and efficient production method of L-glutamic acid, by improving the productivity of L-glutamic acid in a microorganism of the genus Escherichia.

[Constitution]

A method of producing L-glutamic acid by fermenta-

tion comprising culturing in a liquid culture medium a mutant of the genus Escherichia having L-glutamic acid-producing ability whose α -ketoglutarate dehydrogenase activity is deficient or reduced and phosphoenolpyruvate carboxylase and glutamate dehydrogenase activities are amplified, accumulating L-glutamic acid in the culture, and recovering L-glutamic acid therefrom.

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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 95 10 0259

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim							
D,A	FR 2 680 178 A (AJINOMOTO CO., INC.) 12 February 1993 * page 1, line 26 - page 3, line 8 * ---	1,2	C12N15/52 C12P13/14 C12N1/21 //(C12N1/21, C12R1:19)						
A	EP 0 143 195 A (AJINOMOTO CO., INC.) 5 June 1985 * page 1, line 14 - page 4, line 14 * ---	1,2							
A	FR 2 575 492 A (ASAHI KASEI KOGYO KABUSHIKI KAISHA) 4 July 1986 * page 3, line 11 - line 33 * * page 7, line 9 - line 23 * -----	1,2							
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)						
			C12P						
<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 34%;">Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>1 April 1997</td> <td>Montero Lopez, B</td> </tr> </table> <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>				Place of search	Date of completion of the search	Examiner	THE HAGUE	1 April 1997	Montero Lopez, B
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